The dispersions are stable over long periods in distilled water and can be boiled vigorously without ill effect. They are, however, extremely sensitive to electrolytes; for example, the presence of $\sim 10^{-4}M$ Ca^{2+} causes flocculation. Even in distilled water they tend to deteriorate over a period of months owing to displacement of cations such as Mg^{2+} and Al^{3+} from the interior of the silicate layers by protons from the water. The presence of these ions in the "diffuse double layer" upsets the balance of forces operating between particles and leads to gradual flocculation of the system. Addition of acid produces a self-supporting gel at solids contents of the order of 0.25 percent. This gel is nonthixotropic and if broken up does not re-form. Alcohol and other organic liquids miscible with water can be added to lithium dispersions without causing flocculation, provided the dielectric constant of the medium does not fall below about 60. Butylammonium dispersions, however, are more sensitive to the addition of organic liquids and their behavior is less reproducible.

When an aqueous vermiculite dispersion is dried on a smooth surface, a coherent coating is deposited which can be subsequently stripped off the surface as a strong, flexible film. Films a few microns thick can be handled without difficulty. The marked film-forming property of the dispersions in clearly related to the morphology of the vermiculite platelets. When these are deposited by slow evaporation of the water they tend to lie flat on their basal surfaces and overlap with a number of other platelets, allowing strong electrostatic bonding to develop. In their original state the films are highly susceptible to moisture and rapidly disintegrate in water. However, they become insensitive to boiling water or organic solvents if the interlayer cations that produce macroscopic swelling are exchanged for "nonswelling" cations such as Mg^{2+}.

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Amino Acid Coding in Sarcina lutea and Saccharomyces cerevisiae

Abstract. *Aminoacyl-tRNA's from* Sarcina lutea *were tested for incorporation into protein in a heterologous system from* Escherichia coli *or for binding in a homologous system from* Sarcina lutea. *Aminoacyl-tRNA's from* Saccharomyces cerevisiae *were tested for binding in a homologous* Saccharomyces cerevisiae *system. Synthetic polyribonucleotides were used as messengers. The code which exists in* Sarcina lutea *and* Saccharomyces cerevisiae *is the same as in* Escherichia coli.

Amino acid coding has been studied most extensively in vitro with systems derived from *Escherichia coli* (*1*), although a limited number of studies have also been performed on systems derived from other microbial (*2*), plant (*3*), and animal (*4*) cells. The number of different organisms examined is still relatively small and, in addition, most studies have been performed with relatively unpurified subcellular fractions. To evaluate further the universality of the amino acid code in microorganisms other than *E. coli*, systems were prepared from *Sarcina lutea* and *Saccharomyces cerevisiae* for study in vitro. These particular species were chosen because the base compositions of their respective DNA's differ from each other and differ from that of *Escherichia coli*: the content of guanine and cytosine in *E. coli* DNA is 50 percent of the total base content, whereas that of *Sarcina lutea* is 68 percent and that of *Saccharomyces cerevisiae* is 37 percent (*5*). Thus these species might be expected to have different genetic codes if such diversities do in fact exist in microorganisms.

In the first phase of our study amino acid incorporation as a function of various polyribonucleotides was examined in a heterologous system composed of aminoacyl-tRNA from *Sarcina lutea*, and transferase enzymes and washed ribosomes from *Esherichia coli*. To corroborate the data obtained, the binding assay of Nirenberg and Leder (*6*) was employed in a homologous *Sarcina lutea* system. In the second phase of the study the binding assay alone was used in a homologous system derived from *Saccharomyces cerevisiae*. In all cases synthetic polyribonucleotides, but not oligoribonucleotides or triplets, were used as messengers.

Sarcina lutea (American Type Culture Collection 10054) and *Saccharomyces cerevisiae* (ATCC 11795) cells were harvested and washed in Nirenberg's standard buffer (*7*); cell walls were broken in a French pressure cell. After the ruptured-cell suspension was centrifuged, the supernatant was incubated (*7*) and then centrifuged at 100,000*g*. A portion of the supernatant was passed through a Sephadex G-25 column, and fractions with high absorption at 280 mμ were stored in liquid nitrogen. The ribosomal pellets from this centrifugation were washed (*7*), dispersed in 0.01*M* tris-acetate buffer (*p*H 7.8) containing 0.001*M* magnesium acetate and 0.05*M* potassium chloride, and stored in liquid nitrogen. tRNA from *Sarcina lutea* was prepared according to Zubay's procedure (*8*). C^{14}-Aminoacyl-tRNA from this organism was prepared in the presence of 19 unlabeled amino acids by the method of Nirenberg *et al.* (*9*). tRNA from *Saccharomyces cerevisiae* (General Biochemicals, Inc.) was dissolved in 0.1*M* tris-hydrochloride buffer (*p*H 9.0) incubated for 2 hours at 37°C, and deproteinized with phenol. After ether extraction, the tRNA solution was dialyzed against distilled water and freeze-dried. Conditions for "charging" *S. cerevisiae* tRNA with C^{14}-amino acids were essentially the same as those for *Sarcina lutea*. *Escherichia coli* W3100 ribosomes were separated from the supernatant after centrifugation at 100,000g. The ribosomes were washed and the tRNA was prepared as out-

Table 1. Incorporation and binding with C^{14}-aminoacyl-tRNA from *Sarcina lutea*. *Escherichia coli* and *Sarcina lutea* C^{14}-aminoacyl-tRNA was incorporated into protein with *Escherichia coli* ribosomes and transferase enzymes in the presence of synthetic polyribonucleotides. Each 0.10 ml of assay mixture contained C^{14}-aminoacyl-tRNA (1000 count/min) corresponding to the following number of micromoles of C^{14}-L-amino acid and tRNA, respectively; for *Sarcina lutea*: Phe, 7.1, 4800; Arg, 10.4, 7560; Gly, 32.0, 2664; Leu, 12.5, 4360; and for *Escherichia coli*: Phe, 7.2, 5960. In addition, each mixture contained 1.0 A^{260} unit of *E. coli* ribosomes and 1.75 μg of *E. coli* transferase protein. The reactions were incubated for 30 minutes at 37°C. For the binding of *Sarcina lutea* aminoacyl-tRNA to *S. lutea* ribosomes in the presence of synthetic polynucleotides, the amount of C^{14}-aminoacyl-tRNA added to each reaction mixture in micromoles of C^{14}-amino acid and tRNA, respectively, was as follows: Phe, 3.64, 2460; Leu, 8.30; 2880; Arg, 5.36, 3900; Gly, 8.06, 6720; and Lys, 1.01, 6960. In addition each mixture contained 2.28 A^{260} units of ribosomes and 0.03*M* magnesium acetate. Experiments were performed in duplicate. Values in parentheses are insignificant.

Polynucleotide		Incorporation (Δ $\mu\mu$mole)*					Binding (Δ $\mu\mu$mole)*				
		E. coli	*S. lutea*				*S. lutea*				
Base	Ratio	Phe	Phe	Arg	Gly	Leu	Phe	Arg	Gly	Leu	Lys
U		3.82	3.74				1.49	(<0.01)	(<0.01)	0.18	(<0.01)
UC	2 : 1					1.33	1.18		(0.03)	1.25	
UC	1 : 2	3.90	3.26			0.92					
UC	43 : 57		0.92			0.63					
UG	2.8 : 1					(0.02)				0.14	
UG	0.58 : 1				0.44				0.11		
UA	8.6 : 1					0.23				(0.05)	
UCG	3 : 1 : 1			0.06							
UGAC	10.0 : 0.02 : 1 : 1	**4.17**	**2.86**	(<0.01)	0.25	0.92					
A							(<0.01)	(<0.01)	(<0.01)	(<0.01)	**0.11**
AG	1 : 1			(0.04)	(0.03)						
ACG	3 : 1 : 1							0.10			
CG	9 : 1			0.13				0.27			
					Control						
None ($\mu\mu$mole)		1.00	1.20	0.41	1.24	1.55	1.03	0.98	1.32	0.64	0.55

* $\Delta\mu\mu$mole represents the difference between C^{14}-amino acid incorporation into protein (or C^{14}-aminoacyl-tRNA binding) in the presence and absence of polyribonucleotides.

lined above for *S. lutea*. Transferase enzymes were purified from *E. coli* supernatant (100,000*g*) with diethylaminoethyl (DEAE)-cellulose (Whatman) column chromatography according to Nathans and Lipmann (*10*). Incorporation of amino acid into protein was assayed essentially as described by Nirenberg (*7*). Polyribonucleotides were synthesized by Dr. O. W. Jones according to the method of Singer and Guss (*11*) and base ratios were determined by Jones and Nirenberg (*12*). The binding of specific aminoacyl-tRNA to washed ribosomes in the presence of synthetic polyribonucleotides was assayed as described by Nirenberg and Leder (*6*).

In view of limited amounts of *S. lutea* C^{14}-aminoacyl-tRNA's the optimum amounts of aminoacyl-tRNA, washed ribosomes, and transferase enzymes necessary for the *S. lutea* amino acid incorporation system was determined with C^{14}-phenylalanyl-tRNA from *Escherichia coli* and polyuridylic acid in a homologous *E. coli* system. Once these ratios were established, it was assumed that replacement of *E. coli* phenylalanyl-tRNA with *S. lutea* phenylalanyl-tRNA would not grossly affect the in vitro incorporating system. Our data (Table 1) suggest that this assumption was correct for phenylalanine since the degree of incorporation obtained with the heterologous system (that is, *Sarcina lutea* phenylalanyl-tRNA with *Escherichia coli* ribosomes) was very similar to that obtained with the homologous system (*E. coli* phenylalanyl-tRNA with *E. coli* ribosomes). Furthermore, these data suggest that neither the ribosomes nor the transferase enzymes were able to differentiate to any marked degree between the homologous or heterologous aminoacyl-tRNA.

Incorporation of *Sarcina lutea* Phe-, Arg-, Gly-, or Leu-tRNA (*13*) was examined in the presence of several different polyribonucleotides. The amount of the Phe-tRNA incorporated was greater than that obtained with other aminoacyl-tRNA's. Maximum incorporation was observed in the presence of polyU, although substantial incorporation was also observed with polyUC; polyUC (1 : 2) was more effective than polyUC (43:57). The heteropolymer, polyUGAC, which had a high content of U, also gave good stimulation. Arg-tRNA showed a small amount of coding in the presence of polyAG and polyUCG, but was best coded by polyCG which had a high content of C with respect to G. In the presence of polyAG, Gly-tRNA showed little response; the response to polyUGAC was greater, and it was maximum to polyUG that had a high G content. Incorporation of Leu-tRNA was maximum in the presence of polyUG containing different ratios of U to G, much less by polyUA, and very little by polyUG; polyUGAC with a high U and low G content gave good incorporation.

A homologous system from *S. lutea* was prepared and different polyribonu-

Table 2. Binding of *Saccharomyces cerevisiae* aminoacyl-tRNA to *S. cerevisiae* ribosomes with synthetic polyribonucleotides. The amount of radioactive aminoacyl-tRNA added to each reaction mixture, in micromicromoles of C^{14}- or H^3- amino acid and tRNA, respectively, was as follows: C^{14}-Phe, 25.5, 1000; H^3-Leu, 11.8, 2000; C^{14}-Val, 12.3, 1500; C^{14}-Ser, 24.5, 1500. In addition, each mixture also contained 2.44 A^{260} units of ribosomes and 0.04*M* magnesium acetate.

Polynucleotide		Binding (Δ $\mu\mu$mole)*			
Base	Ratio	Phe-	Leu-	Val-	Ser-
U		6.20	0.21	(0.01)	0.19
UC	0.75 : 1	0.07†	0.31	(0.01)	2.01
UG	2.8 : 1	0.32†	0.52	0.61	
None ($\mu\mu$mole)		0.31 0.14†	0.10	0.10	0.18

*$\Delta\mu\mu$mole represents the difference between C^{14}- or H^3-aminoacyl-tRNA binding in the presence and absence of polyribonucleotide. † Results obtained from a separate experiment.

cleotides were tested with various aminoacyl-tRNA's by the binding assay (*6*). Good aminoacyl-tRNA binding was obtained with 30 m*M* magnesium ion, as suggested from studies on *E. coli*. The amount of ribosomes required for optimum binding was determined with polyU and Phe-tRNA and was 1.50 A^{260} units of ribosomes per 25 mμmole of base residues in polyU. This ratio is very similar to that obtained with the *Escherichia coli* system (*6*) although the total number of micromicromoles of Phe-tRNA bound with the *Sarcina lutea* system was somewhat lower (Table 1).

The effect of magnesium acetate concentration on the binding of *Saccharomyces cerevisiae* Phe-tRNA to *S. cerevisiae* ribosomes was studied in a system containing polyU. Binding was quite dependent on divalent-ion concentration and required 40 to 50 m*M* magnesium acetate for maximum results. This concentration is somewhat higher than that required in the homologous *Escherichia coli* system (*6*) where 20 to 30 m*M* magnesium acetate gave optimum binding of Phe-tRNA in the presence of polyU. As expected, binding was not dependent on the addition of GTP. With this optimized *S. cerevisiae* system, the coding of Phe-, Leu-, Val-, and Ser-tRNA was studied in the presence of polyU, polyUC, and polyUG (Table 2).

Variations in template efficiency of different polyribonucleotides, in charging efficiency of different amino acid-specific tRNA's and in other factors such as temperature and cationic environment can all lead to quantitative differences in the degree of incorporation or binding obtained. Furthermore, the use of purified subcellular fractions as well as aminoacyl-tRNA is known to give less incorporation than that obtained with free amino acids and a supernatant from centrifugation at 30,000*g* (*9*). The high "background noise" of the binding assay, even in the *E. coli* system (*6*), also complicates interpretation of the results. Therefore, in the absence of additional control experiments our results alone cannot be used to determine without equivocation new and unique codon assignments for *Sarcina lutea* and *Saccharomyces cerevisiae*. But they can be used to see whether the same codons which were effective in *Escherichia coli* (Table 3) are effective in *S. lutea* and *S. cerevisiae*. In no case was any noteworthy discrepancy found. Those codons expected to code for a particular amino acid did so, and those not expected to code did not.

Except for the extensively studied *E. coli* system, the results of coding studies (Table 3) are probably dependent on the experimental methods used. For example, certain amino acids (leucine through valine) have been more extensively studied than others (alanine through isoleucine), and heteropolymers containing U have been more thoroughly examined than those not containing U. As trinucleotides whose sequences are known become more readily avail-

Table 3. Codon assignments in various species. Order determined only for *E. coli*. In other species, order listed is assumed.

E. coli (*1*)	*S. lutea*	*S. cerevisiae*	*A. faecalis* (*2*)	Mouse ascites leukemia (*4*)	Rat liver (*4*)	Wheat germ (*3*)	*Chlamydomonas* (*2*)
			Alanine				
GCU GCA GCC GGG							
			Arginine				
CGU CGA AGA CGC CGG AGG	CGC		AGA				
			Asparagine				
AAU AAC							
			Aspartic acid				
GAU GAC						GAU	
			Cysteine				
UGU UGA AGU UGC UGG AGC			UGU				
			Glutamine				
CAA CAG			CAA				
			Glutamic acid				
GAA GAG			GAA			GAU(?)	
			Glycine				
GGU GGA GGC GGG	GGU		GGU		GGU	GGU	
			Histidine				
CAU CAC							
			Isoleucine				
AUU AUC			AUU		AUU	AUU	AUU
			Leucine				
UUA CUU CUA UUG CUC CUG	CUU	UUG CUC	UUA CUU UUG	UUG	CUU UUG	UUA CUU UUG CUC	
			Lysine				
AAA AAG	AAA		AAA	AAA	AAA	AAU(?)	
			Methionine				
AUA AUG						AUG	
			Phenylalanine				
UUU UUC	UUU UUC	UUU	UUU	UUU	UUU	UUU	UUU
			Proline				
CCU CCA CCC CCG			CCU CCA CCC			CCU	
			Serine				
UCU UCA AGU UCC UCG AGC		UCC	UCU	UCU	UCU	UCC	UCU
			Threonine				
ACU ACA ACC ACG			ACA				
			Trytophan				
UGA UGG			UGG		UGG	UGG	
			Tyrosine				
UAU UAC			UAU	UAU	UAU	UAU	UAU
			Valine				
GUU GUA GUC GUG		GUU	GUU	GUU	GUU	GUU GUG	GUU

able, the question of codon universality can be more thoroughly and explicitly evaluated by the binding assay. Nevertheless, the various species examined represent an ever-increasing range of evolutionary diversity. Of the 20 naturally occurring amino acids so far studied (Table 3) probably 16 are universally determined by the same code words as found for the respective amino acids when the *E. coli* system is used. Of these amino acids leucine is the only one which clearly shows the unique feature of multiple codons. Further data is required to compare the initiation, termination, and nonsense code words which exist in various phylogenetically divergent species.

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13. Abbreviations used: tRNA, transfer RNA; Phe-, Arg-, Gly-, Leu-, Val-, Ser-, and Lys-tRNA, phenylalanyl-, arginyl-, glycyl-, leucyl-, valyl-, seryl-, and lysyl-tRNA, respectively; U, uridine; C, cytidine; A, adenosine; G, guanosine; tris, tris(hydroxymethyl)aminomethane; GTP, guanosine triphosphate; *A*, absorbancy.
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Hydrocarbons in Digestive Tract and Liver of a Basking Shark

Abstract. *The hydrocarbons of zooplankton pass through the digestive tract of the basking shark without fractionation or structural modification. They are resorbed in the spiral valve and deposited in the liver. In contrast to unsaturated fatty acids, the olefinic hydrocarbons are not decreased in concentration. The hydrocarbon assemblage in the digestive tract and in the liver is indicative of the food sources and feeding grounds of the shark. Squalene, abundant in shark liver, occurs only in traces in zooplankton; phytane, if present at all, constitutes less than 0.005 percent of the hydrocarbons of zooplankton and of shark liver.*

A juvenile male basking shark [*Cetorhinus maximus (Gunnerus)*], 3.9 m, nose to caudal notch, was harpooned off Menemsha, Martha's Vineyard, Massachusetts, in Vineyard Sound on 7 July 1966. The animal was bled and brought into Eel Pond, Woods Hole, Massachusetts, on the same day. It was dissected, and the contents of the digestive tract and several sections from the same lobe of the liver were taken for analysis. Samples for organic analysis were frozen and maintained at −15°C (see Table 1). The basking shark feeds on plankton; therefore, a sample of mixed zooplankton from the Gulf of Maine was analyzed for comparison with the samples from the shark.

The cardiac stomach of the shark contained large quantities of copepods, few larger planktonic animals, and only traces of filamentous algae. Many recognizable specimens of growth stages III and IV of *Calanus hyperboreus* and *C. finmarchicus* were present. Both are abundant at this time of the year in the deeper waters of the Gulf of Maine. A single *Centropages typicus* (adult male) was identified. This neritic copepod is common to the surface waters of the area where the shark was encountered.

The material in the pyloric stomach contained much less water than that in the cardiac stomach. Individual copepods were no longer recognizable. Small amounts of free, red copepod oil appeared in the bursa entiana. No free oil and little extractable lipid material were found in the light tan paste contained in the spiral valve.

The lipids were extracted by homogenizing the samples with anhydrous sodium sulfate under pentane. Fractions (10 μl) of the solvent-free lipids were chromatographed on a 1-ml bed of silica gel (*1*) deactivated with 10 percent water. The saturated hydrocarbons together with the mono-, di-, and triolefins were quantitatively eluted by 1.5 ml of *n*-pentane. This fraction was analyzed by gas chromatography on FFAP (*2*). Duplicate analyses of the same sample of basking shark liver oil (Table 1, last two columns) indicate good analytical reproducibility. Normal heptadecane, whose elution follows the very large pristane peak, is determined with less accuracy than the remaining compounds. Squalene was determined by hydrogenation of the lipids in isooctane with platinum oxide, followed by chromatographic recovery of the saturated hydrocarbons and by gas chromatography as squalane.

To search for phytane, a larger quantity of the hydrocarbon concentrate obtained by chromatography was chromatographed again over activated silica gel (120°C). The saturated hydrocarbon fraction containing pristane and possibly phytane, together with the normal paraffins, was collected and analyzed by gas chromatography. Phytane would have been detected at a ratio of 1 part phytane to 20,000 parts pristane. None was found, either in the oil of the copepods collected in the Gulf of Maine or in the liver oil of the basking shark.

Structures, gas chromatographic retention data, and infrared band positions of the C_{19} monoolefins and of the C_{20} diolefins have been reported (*2*). The structures of the olefins D and E (Table 1) are still unknown. Both compounds have the carbon skeleton of pristane to which they are converted by hydrogenation. Gas chromatographic retention data suggest that compound D is possibly a diolefin, and compound E a triolefin.

The analytical data indicate little or no differentiation between saturated and olefinic hydrocarbons in the digestive tract and in the liver of the basking shark. The drop in concentration of C_{20} diolefins between the pyloric limb of the stomach and the spiral valve is not reflected in the content of C_{19} di- and triolefins. Thus, it may show differences in the food rather than a gradual hydrogenation or destruction of the more unsaturated compounds during digestion. The relative